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Original paper

Bladder cancer is the fourth most common cancer in men and the fifth most common cancer worldwide. UroVysion FISH has high sensitivity and specificity for urothelial carcinoma detection. We investigated the genetic marker detected by the UroVysion FISH technique in diagnosis of Turkish bladder cancer patients and compared these results with the urine cytology and cystoscopy. Urine specimens were analyzed using UroVysion FISH probes for abnormalities in centromeric chromosomes 3, 7, and 17 and locus-specific 9p21.

Morning fresh voided urine samples were collected from each patient for FISH analysis. Cytology and histopathology analysis were performed by the pathology department. Twenty-seven bladder cancer patients (23 male and 4 female) with a history of bladder cancer who provided informed consent were included in this prospective study.

The results showed that cancer was detected in 8 patients via FISH; 7 via cytology; 12 via cystoscopy. According to the pathology results, 15 were normal, 10 high-grade carcinoma and 2 low-grade carcinoma. Sensitivity of these methods with FISH, cytology, and cystoscopy was 29.6%, 25.9%, and 44.4%, respectively.

In conclusion, all tests have different advantages and disadvantages. Also, larger studies will be needed to confirm these results. But, UroVysion FISH appeared to have good specificity for detecting bladder cancer in urine specimens and also it is important to correlate the FISH results with the cystoscopy and cytological findings.

Key words: UroVysion FISH, bladder cancer, voided urine, cytology.

UroVysion fluorescence *in situ* hybridization (UroVysion FISH) assay for detection of bladder cancer in voided urine of Turkish patients: a preliminary study

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Introduction

Bladder cancer is the fourth most common cancer in men. More than 90% of bladder tumors are transitional cell carcinomas. Approximately 80% of transitional cell carcinomas are confined to the epithelium (pTa, CIS) or lamina propria (pT1) at initial diagnosis, but the remaining 20% invade the muscularis propria (pT2, pT3, pT4). pTa lesions (papillary tumors) are the most common form of bladder carcinoma [1]. The most common type of bladder cancer is superficial or invading lamina propria. Patients with superficial bladder carcinoma have a significant risk for recurrence and progression to muscle invasive disease [2, 3].

Fluorescence *in situ* hybridization (FISH) assay is a molecular genetics technique which has been successfully used in cancer studies [4]. But the targets must be highly specific and frequently characterized with abnormalities. UroVysion FISH is a new molecular technique to determine the most common urothelial carcinoma (UC) which is related to chromosomal abnormalities with DNA probes in voided urine [5]. Urovysion FISH has high sensitivity (73–92%) and specificity (89–96%) for urothelial carcinoma detection and several studies have confirmed its efficacy in the diagnosis and surveillance of patients with urothelial carcinoma [6–8]. This genetic test is commonly used for detecting aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus in exfoliated urothelial cells in the bladder. These selected chromosomes correlate with the transition of normal urothelium to carcinoma and also with tumor progression [5, 9]. These chromosome changes are correlated with bladder cancer patients' pathological stages and grades. Chromosomes 3, 7 and 17 polysomies are correlated with high-grade bladder carcinoma and chromosome 9p21 deletions are seen in 60% of superficial low-grade, papillary tumors in bladder [10, 11]. Although UroVysion FISH was initially approved for use in urine samples, it will also be applied to formalin fixed, paraffin-embedded tissues in the future.

In the current study, we investigated the UroVysion FISH technique in the diagnosis of Turkish bladder cancer patients and compared this result with the urine cytology and cystoscopy. Also we assessed the sensitivity of FISH probes with patients.

Material and methods

Patients

Twenty-seven bladder cancer patients were included in this prospective study. Informed consent was obtained from all patients. The study was approved by the institutional review boards. Median age was 66.8 years (range, 49–87 years). Morning fresh voided urine samples were collected from Ege University School of Medicine Department of Urology and FISH analysis was performed and evaluated in the Department of Medical Biology-Genetics. Cytology and histopathology analysis were performed by the Department of Pathology. Clinical follow-up data, including cystoscopy, and cytology findings, were obtained from patients' medical records.

Histopathology and cytological evaluation

Patients were classified according to histopathological staging. Cytology slides were stained using the Papanicolaou method and screened, and a diagnosis of negative or positive for malignancy was rendered.

UroVysion fluorescence *in situ* hybridization assay

During the study period, FISH analysis was performed only when requested by the urologists. From 20 to 50 ml of voided urine were received from each patient. Urothelial cells were centrifuged and first washed with distilled water and then fixed in 3 : 1/v : v methanol : glacial acetic acid. The cell pellet was suspended in fixative and the suspension was applied to a microscope slide [7, 9]. The FISH assay was performed with the UroVysion Bladder Cancer DNA probe kit (Abbott Laboratories, Abbott Park, IL). The probe set consisted of three repetitive sequences recognizing the centromeric regions of chromosomes 3, 7, and 17 and a unique locus sequence that hybridizes to 9p21. These DNA sequences were directly labeled with the fluorophores SpectrumRed, SpectrumGreen, SpectrumAqua and SpectrumGold, respectively. The analysis was performed according to the kit instructions. Approximately 100 cytologically atypical nuclei were scored for the number of fluorescent red, green, aqua and gold signals. An abnormal nucleus was defined as carrying a gain in copy numbers for at least two of the DNA targets or homozygous loss for 9p21 signals.

Analysis of FISH signals

Only non-overlapping cells and cells with distinct signals were scored. The number of signals for all 4 probes was determined and recorded. If chromosomes 3, 7 or 17 demonstrated a loss of both chromosomal signals, the cell was considered to be un-interpretable because of hybridization failure. A cell was considered abnormal if it contained abnormal signals for at least 2 chromosomes. Specimens were considered FISH positive if there were ≥ 4 cells with polysomy for the probed areas on at least 2 chromosomes (3, 7, or 17) and/or 12 cells with no signal for chromosome 9p21. An abnormal specimen was defined as carrying more than 16% of cells with gain for multiple chromosomes or 48% of cells with 9p21 homozygous loss.

Table 1. Patient and tumor characteristics

Characteristic	Median (range) or *n* (%)
Age (years)	66.8 (49–87)
Gender	
male	23 (85.2%)
female	4 (14.8%)
Grade prior to study entry	
low	2 (7.4%)
high	10 (37%)
negative	15 (55.6%)

Tumor stages were not provided for these patients.

Results

A total of 27 patients were enrolled in the present study. Baseline patient and tumor characteristics are presented in Table 1.

Twenty-three of the 27 patients were male and 4 were female and their mean age was 66.8 years (range 49–87).

Table 2. Results of screening strategies for bladder cancer

Patient No.	FISH	Cytology	Cystoscopy	Pathology
1	negative	negative	negative	negative
2	negative	negative	negative	negative
3	negative	negative	negative	negative
4	negative	negative	negative	negative
5	negative	negative	negative	negative
6	negative	negative	negative	negative
7	negative	negative	negative	negative
8	negative	negative	negative	negative
9	negative	negative	negative	negative
10	negative	negative	negative	negative
11	negative	negative	negative	negative
12	negative	negative	negative	negative
13	negative	negative	negative	negative
14	negative	negative	negative	negative
15	negative	negative	negative	negative
16	positive	positive	positive	high grade
17	negative	negative	positive	high grade
18	negative	negative	positive	high grade
19	positive	positive	positive	high grade
20	positive	positive	positive	high grade
21	positive	positive	positive	high grade
22	negative	negative	positive	low grade
23	positive	positive	positive	high grade
24	positive	positive	positive	low grade
25	negative	negative	positive	high grade
26	positive	negative	positive	high grade
27	positive	positive	positive	high grade

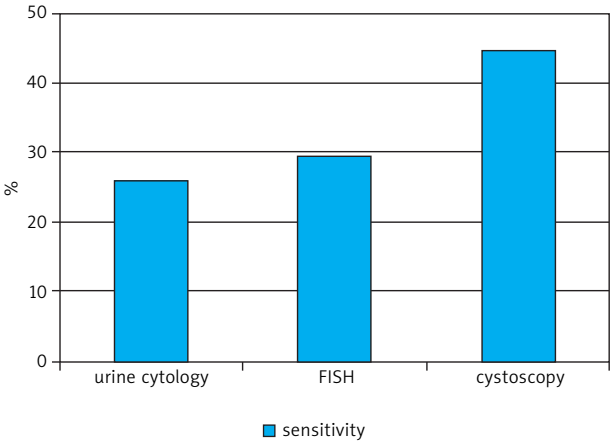

Fig. 1. Diagnostic test results: urine cytology (25.9%), FISH (29.6%) and cystoscopy (44.4%)

The cytology was negative in 20 of a total 27 specimens (74.1%) and positive for malignancy in 7 (25.9%) specimens. FISH was negative in 19 of 27 analyses (70.4%) and positive in the other 8 analyses (29.6%). Cystoscopy was negative in 15 of 27 (55.6%) and positive in 12 (44.4%). Detailed data are given in Table 2. Sensitivity of these methods with FISH, cytology, and cystoscopy was 29.6%, 25.9%, and 44.4, respectively. We have not calculated specificity as we do not have patients without disease. All these patients' urines were taken as they were diagnosed with bladder cancer symptoms.

Seven patients were detected as abnormal with cytology while eight patients were detected as abnormal (positive for cancer) with FISH technique among 27 bladder cancer patients in this study. When FISH results were compared with cystoscopy, 12 patients were positive via cystoscopy whereas 8 patients were positive via FISH analysis. Test performances are shown in Fig. 1. Of the 8 positive FISH analyses, all showed positivity of all analyzed chromosomes; for instance, an interphase cell obtained from a sample showed two copies of chromosome 3 (red), four copies of chromosome 7 (green), five copies of chromosome 17 (aqua) and one copy of the *p16* gene (gold) after the UroVysion Bladder Cancer Kit (Fig. 2). According to FISH technique, seven high-grade and 1 low-grade carcinoma patients showed positive results for cancer (Table 2).

Discussion

The early detection of bladder cancer may affect the local therapies. Cystoscopy is an invasive and expensive procedure for bladder cancer diagnosis. In contrast, cytology of urinary cells is a well-known procedure that has low sensitivity in well or moderately differentiated tumors [12]. An alternative is the use of markers in urothelial cell carcinoma (UCC). Currently, several research groups are working specifically on UCC characteristics and diagnostic approaches. Microsatellite instability, E-cadherin, hypoxia-inducible factor-1α, telomerase, survivin, and multicolor FISH are possible candidates and under investigation [13].

Chromosomal changes are specific for tumor types and are considered critical to the initiation and progression of tumors. Chromosomal abnormalities including aneuploidy have been associated with bladder cancer [14]. Interphase FISH assays have been demonstrated as an effective assay for detection of chromosome aneuploidy [15]. For this purpose, multicolor DNA probe sets have increased the ability

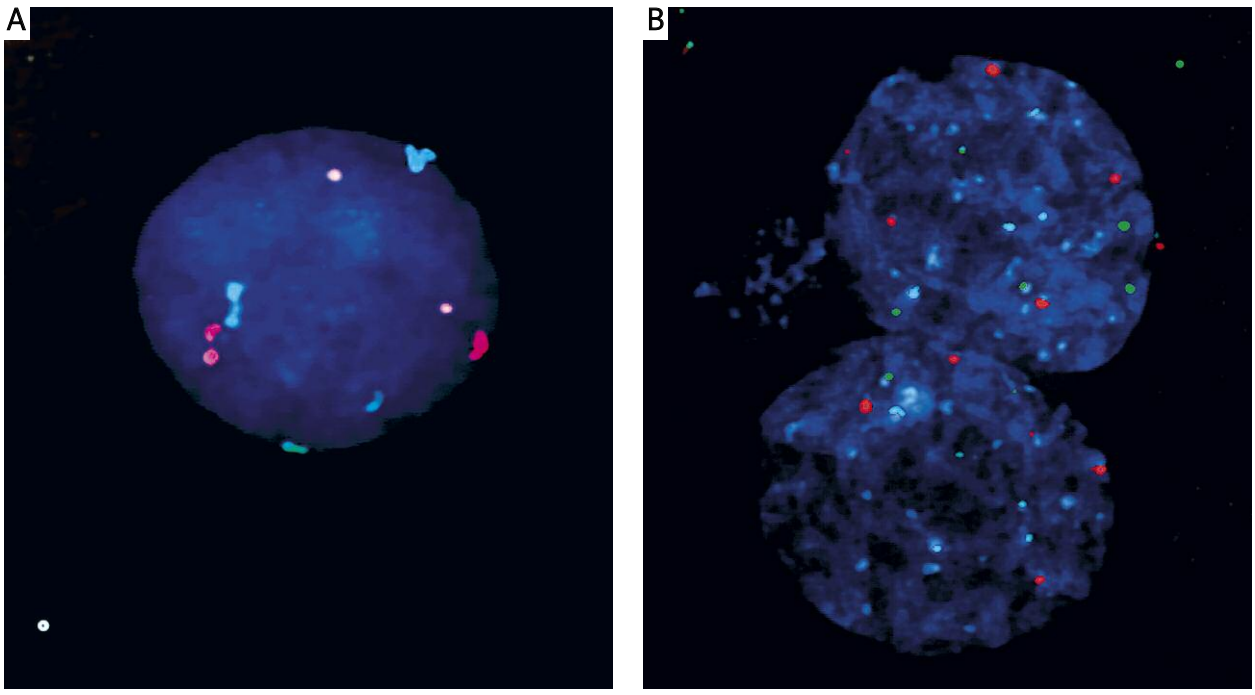

Fig. 2. Aneusomic interphase cell obtained from a sample showing two copies of chromosome 3 (red), four copies of chromosome 7 (green), five copies of chromosome 17 (aqua) and one copy of the *p16* gene (gold) after UroVysion Bladder Cancer Kit (UroVysion Kit) hybridization. **A**) Normal, **B**) Cancer

of testing single interphase cells for numerical chromosomal changes.

The present prospective study focuses on the UroVysion FISH assay which is used to characterize the biological behavior of bladder cancer. First of all, it has been shown that 15 bladder cancer patients could be assessed as negative with a high efficiency by cytology, FISH, and cystoscopy assays (100%).

In the current study, we evaluated bladder carcinoma cases by using UroVysion FISH assay in urine specimens of patients and compared the results with cytology and cystoscopy. Generally, some studies with FISH assay demonstrated a higher sensitivity of UC detection than urine cytology alone [15, 16]. Some previous studies demonstrated that FISH assay had lower sensitivity than cytology technique [3, 9, 17]. Other studies showed that when FISH and cytology assay results were combined, the sensitivity did not show a serious increase [5, 16]. Some factors may contribute to decreased sensitivity in these techniques (low-grade tumors, absence of tumor cells, methods of collection, type of specimen, degenerated cells, etc.).

Some researchers have analyzed genetic abnormalities associated with bladder cancer. Overall, several chromosomal abnormalities have been described in bladder cancer. Previous studies have shown that chromosomes 3, 7, 17 and the 9p21 locus have a high sensitivity and specificity for detecting UC in voided urine samples [2, 6, 18] including bladder cancer.

Previous studies found that the sensitivity of FISH for urothelial carcinomas ranged from 65% to 100% of the tumor and the specificity of FISH in the detection of urothelial carcinomas reportedly ranged from 77% to 97% [17, 19–22]. In this study, we have 29.6% positive FISH results and 70.4% negative FISH results in patients. The cancer detection rates were 25.9% by cytology, 29.6% by FISH, and 44.4% by cystoscopy. On the basis of our findings, cystoscopy has still improved sensitivity.

When considering this decreased sensitivity of FISH compared with the other studies, it should be borne in mind that we have a small series of patients. But this is also a preliminary study, so this will be beneficial to make a new project with large groups.

In conclusion, all tests have different advantages and disadvantages. Also, larger studies will be needed to confirm these results. UroVysion FISH is a useful assay for bladder cancer patients and appears to have slight sensitivity for detecting bladder cancer in urinary samples. As these assays are further characterized, their potential to be helpful ancillary tools in the detection of recurrent urothelial cancer will be further clarified. The present study has potential limitations. The number of patients was low. We think that UroVysion FISH appeared to have good sensitivity for detecting bladder cancer in urine specimens. But, it is important to correlate these FISH results with the cystoscopy and cytological findings in more patients and subgroups.

The authors declare no conflict of interest.

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